STUDIES IN THE AUSTRALIAN ACACIAS. III.

SUPPLEMENTARY OBSERVATIONS ON THE HABIT, CARPEL, SPORE PRODUCTION AND CHROMOSOMES OF ACACIA BAILEYANA F.V.M.

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(Plate vii; thirty-three Text-figures.)

[Read 25th July, 1934.]

The first part of this enquiry (Newman, 1933) described the ecology, habit, floral structures, reproductive processes as far as the production of the spores, and the haploid chromosome number. As the material for that paper was collected late in the season, it did not show some of the earlier stages of development. Good material became subsequently available, so missing stages can now be presented, with further discussion and with additional observations on the chromosomes.

Annotations for the figures have been standardised and will be found in the "Notes on the Illustrations".

Most of the material used for this part of the work was collected by Mr. J. Stewart from trees in cultivation at Strathfield, Sydney. The writer expresses his thanks for good service rendered.

Methods and fixatives were described in the previous paper (p. 146). The alcoholic fixatives were more suitable from the point of view of sectioning as they seemed to make the air more easily extracted from the spaces within the flower heads.

HABIT.

On page 150 of Part I, it was stated that there seemed to be two sections of the species, one with mostly three and one with mostly four pairs of pinnae. A slight statistical examination was made of eight trees in 1932. Though it showed the possibility of a third section with mostly five or six pairs of pinnae, the distribution of the numbers among the samples indicated that there may be a significant variation from year to year, due rather to fluctuations of vigour than to genetical differences. Kelly (1912, p. 120) gives equally wide variations for the size and outline of the phyllodes of other unsplit species of *Acacia*. It is significant, however, that Clos (1929), describing *A. Baileyana* as cultivated in Argentina, refers only to two or three pairs of pinnae. The inference is that the original plant or plants introduced there possessed such a *genetical* character. Before any valid division of the species into sections based on these characters could be made, an accurate statistical examination would have to be undertaken. A similar situation arises with the occurrence of the racemes singly or in pairs.

At the beginning of anthesis the style (contorted in the bud) emerges between the separating tips of the petals. A flower head may have all its styles extended, without any sign of the stamens. The stamens are extended by the lengthening and uncoiling of the filaments, and the tip of the style projects beyond them (Plate vii, fig. 1).

As described in Part I, p. 153, and below, the legume is a folded foliar structure, with an elongated tip of which the stigma is the slightly opened end. The stigma appears as a shallow cup (Plate vii, figs. 2, 3). Note that the heavy cuticle (with wavy surface) on the epidermis of the top of the style does not extend on to the stigmatic surface (Plate vii, fig. 3, *ct.*).

The Morphology of the Legume.

The youngest fertile legumes described in Part I were at the stage of the primary megasporogenous cell. Sections of legumes at a stage earlier than that are shown here in Plate vii, figs. 9–12. The two epidermes of the appressed margins of the folded foliar structure are clearly visible on one side of the carpel, while absence of such features and the presence of the midrib primordium are equally visible on the other side. In *A. Baileyana* the carpel definitely arises as a single folded (foliar) structure.

By the theory of Carpel Polymorphism, Saunders (1925, p. 142) interprets the legume as composed of two carpels and gives *A. suaveolens* and *A. longifolia* as examples (1929, pp. 225–8, 258). After examining microtome sections of the developmental stages of the legume from its initiation to the time of fertilization, it is hard to understand how such an interpretation could be made. This question was discussed in Part I (p. 155); but in view of the additional evidence now available further comment will be made here.

Almost every author who has occasion to describe or figure the legume before post-fertilization development represents it as a single folded structure at whose appressed margins the ovules arise, e.g., Reeves (1930*b*, fig. 2) shows the appressed epidermes in *Medicago sativa*; Taubert (1894, pp. 84–6) figures the single folded structure for the legume in general, as does Thompson (1931) for species of all sections of the Leguminosae; and Bugnon (1925*a*) shows the legume of *Lathyrus vernus*, *Trifolium pratense* and *Lupinus perennis* to be open on one side in the early stages. For comparison, we could note that Brough (1933, pp. 37, 41) describes an almost identical form for the gynoecium of *Grevillea robusta* (Proteaceae). Bugnon undertook his enquiry on account of Grégoire's (1924) statement that the legume in the Papilionaceae is not a folded structure because its primordium is annular and it dehisces along two sides, figuring *Lathyrus*. Grégoire's figures are unconvincing because, though they show tissue structure, their ovules are of such a size and outline that one expects to see differentiation of sporogenous tissue, but does not see it. Moreover, as Bugnon points out, the carpels shown are too old for reference to primordia. It is on account of neglecting truly primordial stages, and emphasizing adult stages, secondary developments and external contour that the misinterpretation of the legume has been possible.

Post-fertilization development of the legume of *Acacia Baileyana* rapidly masks the single and folded nature of its primary morphological structure. It has been seen to have the form of a single folded foliar organ, with appressed epidermes of the margins, with two marginal vascular bundles "adaxially" and the single midrib "abaxially". The carpel is so undeveloped by the time of

fertilization that the vascular bundles are scarcely more than primordial. On the marginal side, the side of the insertion of the ovules, secondary growth produces a narrow zone of parenchyma along the line of the appressed epidermes (*pce.* in Plate vii, figs. 4, 5). On the midrib side secondary growth produces a zone of parenchyma (*pcm.* in Plate vii, figs. 4, 6) from the edge of the loculus to the outer epidermis, dividing the midrib. There is no difficulty in accepting this division of the midrib which, before the secondary growth began, was almost primordial. Between the seeds, the walls of the loculus are pressed together and its epidermal cells become much enlarged and pithy. Along the broad axis (A to B in Plate vii, fig. 4) of the legume, there is a line of weakness composed of parenchyma cells which, except at one end (the midrib end), are mainly cells of two appressed epidermes. There is nothing in this secondary development to refute the previous conclusion that this legume is a single carpel. This investigation emphasizes the necessity of the study of primordial stages in the interpretation of floral structure.

Except where passing between the hard tissues of the vascular bundles, the line of weakness (parenchyma) described above has on either side a zone of collenchyma cells produced by the secondary growth (*cc.* in Plate vii, figs. 4–6). Dehiscence is brought about by the differential contraction of these tissues, and is in no way an indication of the fundamental structure of the legume.

The disputed theory with regard to the legume rests largely on the relative prominence of the secondary vascular systems arising from what are usually regarded as the marginal bundles. Bugnon (1925*b*) contests the application of the theory of Carpel Polymorphism to the legume, objecting to interpretations based on the adult gynoecium without taking into account ontogeny or comparative foliar morphology. He points out that in some Monocotyledons the marginal veins of some floral leaves give off secondary veins while the midrib remains simple. And Arber (1933, p. 235) says that "the predominance of the marginal regions in the carpel as compared with the foliage leaf . . . is not an absolute change, but merely a *variation in relative emphasis;*". She also (p. 233) attacks the assumption in the theory that a vascular system can remain as a survival of an organ of which no external trace exists, holding that the vascular system is rudimentary to the same degree as or more than the rudimentary external form. The case of *Acacia Baileyana* gives general support to this objection in that the marginal bundles receive emphasis only in accordance with the need they serve; for in the fertile carpel they do not pass into the style (i.e., beyond the ovules they supply), and in the sterile carpel without even rudimentary ovules they are entirely absent.

Sporangial Structures.

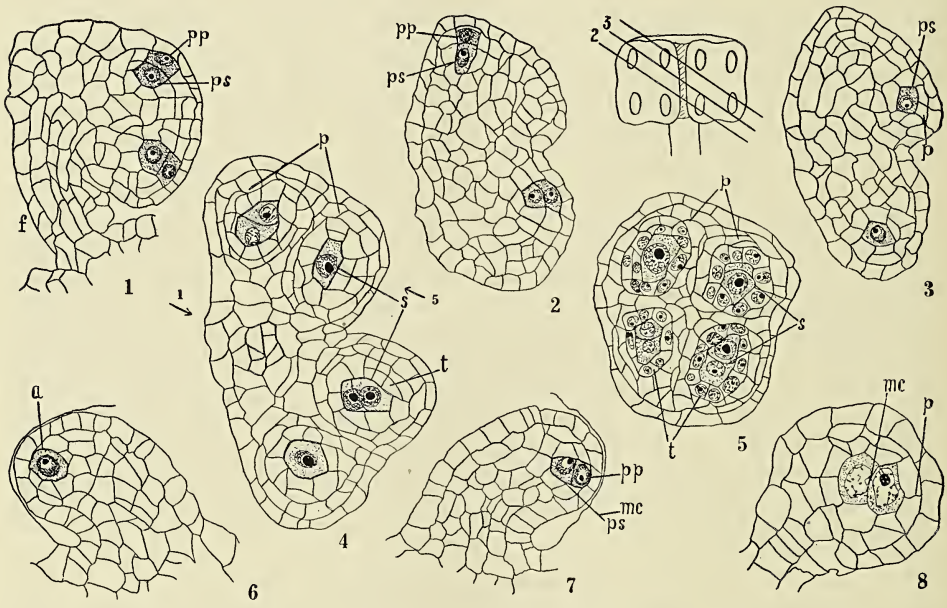
Sporangial structures are considered only up to the shedding of the pollen in the one case and to the time of fertilization in the other case. The sporogenous tissue after the archesporium is considered under "Sporogenesis".

Microsporangial.

The disposition of the eight groups of sporogenous cells in the anther has been described already in Part I, p. 157. Judging from the clear cases of the immediate products of its division (Text-figs. 1, 2), the archesporium is regarded as a single hypodermal cell for each sporogenous tissue (microsporangium). Text-figures 1 and 2 may suggest that the functional archesporium is

selected from a group of archesporial cells. But in view of the smallness of the anther it is considered that there is difficulty in distinguishing somatic meristem from sporangial meristem. The anther is, therefore, held to contain eight morphologically superficial sporangia derived from eight separate archesporia. The whole of each sporangium is shed as a unit, the pollinium. (See also section on "Sporogenesis" below and in Part I, p. 159.)

Albizzia lebbek, in another genus of the Mimoseae, is described by Maheshwari (1931, p. 244) as having a deep-seated archesporium, i.e., with a morphologically embedded sporangium. He says that the archesporium consists of a row of two cells. After comparing his figures with the account given below, it would seem that what he identifies as a row of two archesporial cells is the two cells resulting from the division of the primary sporogenous cell. Compare his figures 4 and 5 with Text-figures 3–5 of this paper. Probably in both *Acacia Baileyana* and *Albizzia lebbek* the early stages are passed through very quickly. In *Asclepias cornuti*, another plant that forms a pollinium, but with many grains in it, the archesporium is a transverse row of cells (Gager, 1902, p. 129).



Text-figures 1-5.—Stages in the formation of the microspore mother cells. × 390. 1, longitudinal section of a young stamen. 2 and 3, consecutive oblique sections of a young anther (with primary sporogenous cells) as in the diagram between the figures. In two cases the primary parietal cell has divided. 4, transverse section of an anther showing parts of four bi-cellular sporogenous tissues, each surrounded by its tapetum. The arrows indicate the plane of sections 1 and 5. 5, longitudinal section of a lobe of an anther showing parts of four bi-cellular sporogenous tissues.

Text-figures 6 and 7.—Longitudinal sections of two young ovules showing the initiation of the sporangium. × 390.

Text-figure 8.—Oblique longitudinal section of a young ovule showing two mother cells in synapsis, possibly derived from one archesporium. × 540.

There are three layers of anther tissue vertically, and four or five layers horizontally between the dividing primary sporogenous cells in *Acacia Baileyana* (Text-figs. 1, 4, 5). From these and the parietal tissue, the tapetum is organized as a (usually) single layer of uninucleate cells for each sporangium, leaving one layer vertically and two or three layers horizontally of non-tapetal cells between the sporangia (Text-figs. 4, 5).

Schürhoff (1926, p. 239) derives the tapetum on the outer side from the primary sporogenous layer (the inner layer resulting from the division of the hypodermal layer). But in *Acacia Baileyana* it is derived from the primary parietal cell. A similar origin for the tapetum has been found in other Leguminosae by Reeves (1930*a*, p. 30) in *Medicago sativa*, Weinstein (1926, p. 250) in *Phaseolus vulgaris*, Maheshwari (1931, Figs. 4, 5)—judging by his figures—in *Albizzia lebbek*, and by Sethi (1930, p. 128) in *Cassia didymobotrya*; in another pollinium-forming genus by Gager (1902, p. 129) in *Asclepias cornuti*; in two other Australian Angiosperms by Brough in *Dampiera stricta* (1927, p. 478) and *Grevillea robusta* (1933, p. 49).

After examining 43 species from 24 families of Angiosperms, Cooper (1933, p. 359) says that they can be divided into three slightly overlapping groups having the following tapetal conditions: 1, uni-nucleate cells; 2, bi-nucleate cells; 3, pluri-nucleate cells. Among the Leguminosae he listed 4 species of *Medicago*, and 2 species of *Melilotus* in group 1, to which can be added *Albizzia lebbek* (Maheshwari, 1933, p. 245) and *Acacia Baileyana*. *Cassia didymobotrya* (Sethi, 1930) can be placed in group 3, as also a pollinium-forming plant from another family—*Asclepias cornuti* (Gager, 1902, p. 130). It is interesting to find that species of the Papilionaceae and Mimoseae are associated in group 1, while a Caesalpineous species is in group 3, otherwise than might be expected in view of the floral forms of these sub-families.

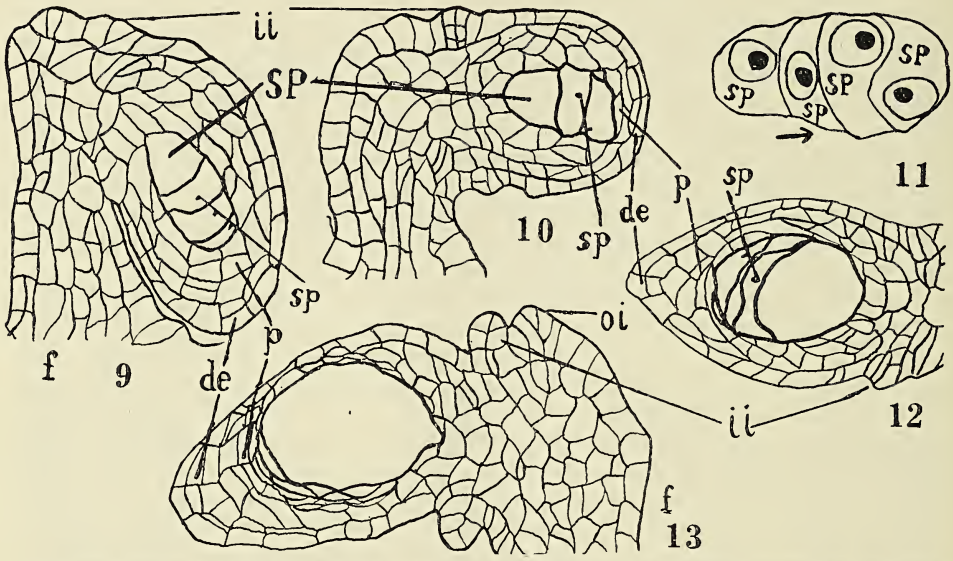
Megasporangial.

In describing the ovule, the term "sporangium" will be used only of the products of the archesporial cell. These comprise the parietal tissue (if any) and the sporogenous tissue (usually one cell only). The sporangium is borne on a receptacle (nucellus) at the margin of the carpel. The term "nucellus" itself will be used only where the whole of the structure above the stalk (funiculus) of the receptacle is referred to, irrespective of the internal differentiations. (For explanation of the use of these terms see Newman, 1928, pp. 515, 522).

From the transverse section shown in Plate vii, fig. 9, it will be seen that the young carpel before the initiation of the ovule consists of three layers of cells surrounded by an epidermis and containing a midrib primordium. Figure 10 on the same plate shows that there are still only three layers within the epidermis, except at the margins, when the ovule primordia appear by multiplication of the hypodermal layer. Though the primordia appear to be on the inner (adaxial) surface, from the curve of the cell layers they can be interpreted as on the morphologically outer (abaxial) surface of the carpel. This depends on whether the margin of the carpel is at B or A in Plate vii, fig. 10. The latter condition was demonstrated by the writer in the case of *Doryanthes excelsa* (Newman, 1928, p. 507).

The megasporangium is derived from a hypodermal archesporial cell (Text-fig. 6) that gives rise to the primary parietal cell and the primary sporogenous cell (Text-fig. 7) whose further development has been described in Part I (p. 162).

The ovule is more or less anatropous, and is naked until after fertilization, the integuments being only primordial collars till then. The inner integument is the first to appear about the time of megaspore formation (Text-figs. 9, 10, 12, 13). The development of the integuments will be described in connection with the development of the seed in a subsequent paper.



Text-figures 9, 10, 12, and 13.—Longitudinal sections of young ovules at various stages. The chalazal megaspore is functioning. × 455. 9, four spores. 10, three spores. 12, two-nucleate sac. 13, organization of cells in the sac.

Text-figure 11.—Row of four megaspores with the two distal spores functioning. × 880.

In Part I, p. 159, it was said that there is no multiplication of the epidermis of the ovule. This is true only until the formation of the spores. After that time periclinal divisions occur in the epidermis over the parietal tissue (Text-figs. 9, 10, 12) till, by the time of fertilization, it is several layers thick (Text-fig. 13, Plate vii, fig. 13).

The epidermis of the chalazal region is continuous with several layers of cells in the distal region of the ovule. By the time of fertilization or very soon after, the only tissue left above and beside the embryo sac is of epidermal origin. This is a correction of the previous statement of absence of crushing of the parietal tissue (loc. cit.).

Starch is plentiful in the nucellus at the time of fertilization. The refringent bodies shown in Plate vii, fig. 13, are the starch grains.

The origin of the ovule from the hypodermal layer appears to be normal (Coulter and Chamberlain, 1903, p. 53). Guignard (1881, p. 28) describes the ovule of the Acacias as completely anatropous at the time of fertilization (based chiefly on *A. retinoides*), and says that the ovules of the Caesalpineae are less anatropous than those of the Mimoseae (p. 45). If this be so, there is again a character common to the Papilionaceae and Mimoseae differing from that possessed by the Caesalpineae which manifest the more primitive condition

(Coulter and Chamberlain, 1903, p. 56). But *Acacia Baileyana* appears to be indefinite in this respect.

The position of the archesporial cell is as generally found in other Leguminosae. Guignard (1881, p. 22 et seq.) described it and its division to primary parietal and sporogenous cells in a number of species of *Acacia*, as did Maheshwari (1933, p. 246), in *Albizzia lebbek*. Among some Papilionaceae, there is a tendency to have a multi-cellular archesporium. Reeves (1930*b*, p. 240) and Martin (1914, p. 160) found mostly two or more archesporial cells in *Medicago sativa*, though Guignard (1881, p. 119) found only one in *M. arborea*. Martin found that sometimes the archesporial cell functioned as the mother cell. In *Trifolium pratense* he also found (p. 156) more than one archesporial cell to be usual. Saxton (1907, p. 1) was not able to recognize an archesporial cell in *Cassia tomentosa*, stating that the mother cell is differentiated deeply. A multicellular archesporium can be considered primitive and is characteristic of the Rosaceae (Coulter and Chamberlain, 1903, pp. 58–61). Here again the Papilionaceae manifest the primitive condition; but in this case the Mimoseae (see also Guignard, 1881, pp. 22, 46) are not associated with them and manifest the advanced condition, in association with the Caesalpineae.

Among the Leguminosae there is considerable variation in the degree of development of the parietal tissue. Interpretation is complicated by the frequent multiplication of the epidermis covering it, from about the time of spore formation. Warming (1878, p. 228) and Vesque (1879, p. 277) have described this multiple epidermis for a number of Angiosperms distributed mostly among the Archychlamydae, but also among the Metachlamydae and the Monocotyledons. Guignard (1881, p. 23) records the multiple epidermis over the parietal tissue as common in the Leguminosae, and Péchoutre (1902, p. 154) does the same for the Rosaceae.

The last three authors are agreed that the multiple epidermis persists usually for a long time, even though all the underlying parietal tissue may be destroyed by the enlarging embryo sac. Martin (1914, figs. 8–12) shows few divisions in the epidermis for *Trifolium pratense*, but this epidermis may even be crushed by the mature embryo sac. Vesque (1879, p. 279) points out that when a more or less massive parietal tissue has a multiple epidermis, destruction of tissue stops short of that epidermis.

Sporogenesis.

Relative Size of Spores.

The question of the relative size of the megaspores and microspores was raised by Gates in a letter to *Nature* (1932*a*). A few measurements in *A. Baileyana* suggest that its megaspore is the smaller. In such a question the spores must be measured at comparable stages. In this case the measurement was made just after the spores were formed.

Microspore.

The eight single hypodermal archesporial cells give rise to the eight single primary sporogenous cells. Two of these, with the primary parietal cells, are shown in Text-figure 1, representing a longitudinal section of a young stamen. Text-figures 2 and 3 represent oblique transverse sections of an anther showing the same and slightly later stages. The primary sporogenous cells are slightly larger and more densely cytoplasmic than the surrounding cells whose meri-

stematic condition makes difficult the identification of the young primary sporogenous cells. But they can be identified by following back to them from the unmistakable condition shown in Text-figures 4 and 5, where can be seen the products of their division both resting and preparing for the second division. This produces the four mother cells in each sporangium. From this stage the account in Part I began.

The occurrence of only four mother cells in the microsporangium makes *A. Baileyana* (and probably all Acacias) suitable material for testing the recent theory of Huskins (1932) that meiosis is initiated by the suppression or retarding of the split in the chromosomes during the last pre-meiotic mitosis. This theory was discussed in a letter to *Nature* (Newman, 1932) in which it was pointed out that an explanation would be needed as to why meiosis did not occur in the primary parietal cells of plants whose primary sporogenous cells functioned as mother cells. For in the anther of *A. Baileyana*, both cells formed by the last pre-meiotic mitosis undergo meiosis. But in ovules such as that of *A. Baileyana* and in anthers such as that of *Grevillea robusta* (Brough, 1933, p. 49) where the primary sporogenous cells function as mother cells, only one of any two nuclei formed by the last pre-meiotic mitosis undergoes meiosis. Huskins (1932, p. 26) reports that he has observed pairing of chromosomes in the parietal tissue in the ovule of *Matthiola incana*. If sometimes, why not always?

Though tetrad formation is not considered here, it is worth noting that Kanda (1920, p. 60) records two types of tetrad formation in *Verbena*: 1, some peripheral cytoplasm of the microspore mother cell remains to form a temporary common wall for the tetrad; 2, all the cytoplasm of the microspore mother cell is used up within the spores. It seems possible that type 1 is a step towards the evolution of pollinia such as occur in the Acacias.

Megaspore.

Megaspore formation has now been found to be succedaneous. Plate vii, figure 15, of the homotypic division, shows that a wall had been formed at the end of the heterotypic division. It is also to be noted that the spindles of the homotypic division are sometimes inclined to one another, which may account for the occasional attempts by two spores to function in the one ovule (Plate vii, fig. 15).

An unusual case of two mother cells in synapsis in one ovule was found (Text-fig. 8). This differs from the case described in Part I, p. 158, in that there is here no tissue between the mother cells, which suggests the possibility of their origin by division of a primary sporogenous cell, a different condition from that of the multicellular archesporium of the Rosaceae.

The functional spore may be proximal (Text-figs. 9, 10, 12) or distal (Plate vii, fig. 14, and Text-fig. 11), but it is difficult to say which is the more frequent. The functioning of the second or third spore is rare.

Several cases of more than one mother cell in an ovule and even more than one sac in an ovule have been seen in *A. Baileyana*, and Guignard (1881, p. 37) records examples of two sacs in an ovule in several species of *Acacia*. No example has been seen in this species of more than one tetrad in an ovule. Therefore, the occasional occurrence of more than one functional spore or sac in an ovule would appear to be due to development of more than one spore of a tetrad. Reeves (1930*b*, p. 241) records that more than one tetrad in an ovule may be formed in *Medicago sativa*, but only one megaspore develops to a complete

embryo sac. The same author (p. 241) records vertical division of the upper daughter cell of the heterotypic division as in the figure given here (Plate vii, fig. 15).

Guignard (1881, pp. 136–7) records, in the Leguminosae, most of the possible variations in the numbers of megaspores formed from one mother cell, namely: the mother cell functioning as spore, two, three unequal, three equal, four equal spores. He listed Mimoseae and Caesalpineae in only the last two groups. Schürhoff (1926, p. 574), summarizing work on the Leguminosae, says there are three or four spores of which the lowest or second lowest functions. Martin (1914, p. 160) records occasional functioning of the third megaspore in *Trifolium repens*. *A. Baileyana*, therefore, with the approximately equal functioning of the chalazal or distal megaspore, seems to occupy a unique position among the Leguminosae. In another Australian Dicotyledon, *Styphelia longifolia* (Epacridaceae), it is the distal megaspore that functions (Brough, 1924, p. 168). According to Saxton (1907, p. 2) the non-functioning of the proximal megaspore among the Dicotyledons is almost confined to the Rosaceae and the Leguminosae.

Sterility.

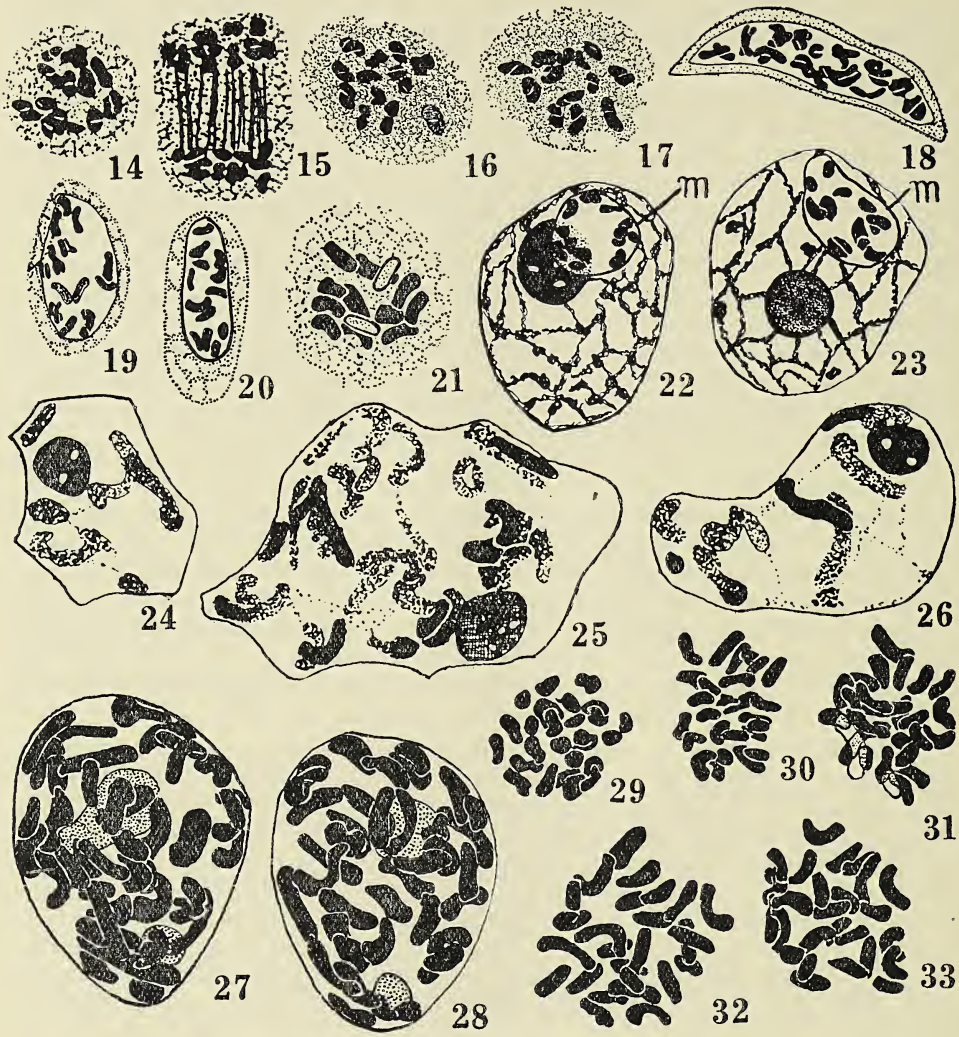
Sterility in the female organs seems to be influenced considerably by geographical position. For, at Hill Top, about 90 Km. south-west from Sydney and at an altitude of 600 m., the setting of the seed is abundant, while at Strathfield, Sydney (at altitudes up to 50 m.) it is difficult to get material giving preparations of fertile ovules in the few fully developed legumes produced (Plate vii, figs. 7, 8). Note that the ovules shown in Figure 8 had attained full size before degenerating. In contrast to the above, copious germination was obtained in culture of pollen collected at Strathfield.

Maheshwari (1931, pp. 251, 260) describes degenerations in *Albizzia lebbek* only after formation of the microspores in the anther, and in the ovule only from the prophase of meiosis onwards—mostly after the formation of tetrads—and concludes that it represents "an inherent tendency towards the elimination of one or both of the sex organs on some flowers". In *Trifolium pratense*, Martin (1914, p. 159) finds that sterility occurs before the formation of mother cells, as none of these were found in synapsis in sterile ovules. The general and indefinite sterility in *A. Baileyana* seems in a different category from these types referred to, nor is it a Mendelian character such as described for pollen sterility (confined to mother cells) in some races of *Lathyrus odoratus* by Gregory (1905, p. 154).

Chromosomes (*Number, Form and Behaviour*).

Shimotomai (1933, p. 4), working with the genus *Chrysanthemum* in which there is much polyploidy, avoids the difficulty of confusing the "haploid" and "diploid" numbers of different species by reserving the letter "n" for the basic number of chromosomes for the genus, using "ϕ" for the gametic number and "2ϕ" for the zygotic number. This convention will be used in the study of the Acacias from now on.

The gametic number, $\phi = 13$, was counted previously for *Acacia Baileyana* in meiosis in the anther (Newman, 1933, p. 166). This number has now been confirmed by counts in microspore division, the generative nucleus, the male nuclei within the embryo sac, the homotypic division in the ovule and the second and third divisions in the embryo sac (Text-figs. 14–23; Plate vii, figs. 15–17*b*').



Text-figures 14 to 33.—Chromosome numbers and forms. In these figures the presence or absence of trabants is disregarded. × 3250.

Text-figures 14-23.—The gametic complement of chromosomes, ϕ = 13, in various situations. Note the size differences and the frequent visibility of the split at anaphase and telophase. 14 and 15, metaphase and anaphase of microspore division. 16 and 17, polar view of the two groups of an anaphase of microspore division; note the corresponding positions of the chromosomes in the two groups; the lighter chromosome was on the next section. 18, young generative cell with telophase chromosomes. 19 and 20, generative cell in the pollen grain and tube respectively. 21, metaphase of the third division in the embryo sac, polar view. 22 and 23, male nuclei (m) in contact with egg and polar nuclei respectively.

Text-figures 24-28.—Endosperm nuclei in prophase. Showing approximately 3ϕ = 39. Note the size differences in the chromosomes. 24-5-6, consecutive sections of a primary endosperm nucleus distorted by starch grains. 27-8, daughter nuclei of the primary endosperm nucleus.

The zygotic number, $2\phi = 26$, has now been counted in the following tissues (Text-figs. 29–33): filament, microsporogenous tissue, inner and outer epidermis of the pod, and the testa of a young seed. In these zygotic counts trabants and constrictions were disregarded.

The endosperm number, $3\phi = 39$, has been observed, approximately, in the primary endosperm nucleus and its daughter nuclei (Text-figs. 24–28).

Hedayetullah (1931) in *Narcissus*, and Koshy (1933, p. 304) in *Allium* have described the anaphase chromosomes as showing the split for the next mitotic division. This split is very clearly shown in some of the anaphase and telophase figures given in this paper (Text-figs. 15–18; Plate vii, figs. 15, 17*a*-*b*′). The split for the current division is shown in prophase nuclei of gametophytic tissue (Text-fig. 19; Plate vii, fig. 16, *a*, *b*).

In Plate vii, figures 16*a*-17*b*′, of prophase and telophase of the second and third divisions respectively in the embryo sac, there are shown structures that strongly suggest trabants (see Part I, pp. 160 and 166).

There are marked differences in chromosome size. I am of the opinion that in the gametic complement there is one chromosome definitely larger, and three chromosomes definitely smaller than the others. One or both of these features are to be seen in most of the figures showing chromosomes. It is remarkable that such a feature should be observable, not only in divisions in the formation of the embryo sac, but also in the generative nucleus and the male nuclei. The same differences with the numbers doubled are discernible in the figures from zygotic tissue (Text-figs. 29–33).

A detailed study of the cytology of *Acacia Baileyana* and other species will be made at a later date; but certain points may be mentioned now. It was pointed out in Part I (p. 167) that 13 was a recent discovery for the gametic chromosome number among Leguminosae, the usual numbers being low multiples of 6, 7, and 8. Evidence is accumulating that a number such as 13 may be a secondary polyploid number based on a simple number such as 6, 7, or 8 with duplication of one chromosome or fusion of one or more pairs of chromosomes. For instance, in *Cassia occidentalis*, $\phi = 13$, Muto (1929, p. 270) figures two, possibly 3, distinctly large chromosomes and in *C. didymobotrya*, $\phi = 14$, Sethi (1930) finds no very large chromosomes. Other plants with 13 as a basic number for the chromosome complement are *Epacris impressa* (Samuelsson, 1913) and species of *Gossypium* (Denham, 1924; Banerji, 1929; Davie, 1933; Skovsted, 1933). All four authors agree in finding two races of *Gossypium*, the Asiatic species with $\phi = 13$ and the New World and Egyptian species with $\phi = 26$. Compare the similar difference between Australian and extra-Australian species of *Acacia* (Ghimpu, 1929, *a*, *b*). Skovsted (pp. 243–4) and Davie (pp. 44–45) discuss the possibility of these species of *Gossypium* being secondary polyploids based on lower numbers with duplication or fusion of chromosomes, Skovsted also considering the possibility of polyploidy based on the numbers 7 and 6. If fusions are the case, then Davie finds the appropriate number of large chromosomes to be present. Gates (1932*b*, p. 10) has quoted records of end to end fusion of two chromosomes to form a

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Text-figures 29-33.—Polar view of zygotic chromosome complements showing $2\phi = 26$. Note the size differences of the chromosomes. 29, anaphase group from the filament of a stamen. 30, anaphase group from the division of a primary sporogenous cell. 31, metaphase from the epidermis of the loculus of a young pod; the two chromosomes in outline were on the next section. 32, metaphase from the outer epidermis of a young pod. 33, metaphase in the testa of a young seed.

single body. If the basic number, n = 13, for the Acacias is derived by the fusion of two chromosomes to form one large one, then the appropriate numbers of large chromosomes are considered to be present in *Acacia Baileyana*, and there is an approach to these numbers in the illustrations given by Ghimpu (1930, p. 192, 198) for mitosis in *A. cyanophylla* and *A. scorpioides* var. *adstringens*. It seems reasonable to suggest that the number ϕ = 13 for *A. Baileyana* is secondary polyploidy based on duplication of the number 7 with fusion of a pair of chromosomes to form one large one. The investigation of the cytological details of *Acacia Baileyana* will soon be undertaken.

Summary and Conclusion.

This paper presents supplementary observations and discussion on vegetative features, spore production and chromosomes of *Acacia Baileyana*.

After slight examination, it is concluded that extensive statistical inquiry would be necessary before splitting the species into sections based on the numbers of pinnae.

Anthesis with proterogyny is described.

The primordia of the legume are described and discussed in refutation of the application of the theory of Carpel Polymorphism to the legume. The post-fertilization development of the pod is shown not to support that theory.

In the anther, there are 8 separate unicellular archesporia. The tapetum is derived from the primary parietal cell; and its cells are uninucleate, being associated therein with the Papilionaceae and not with *Cassia didymobotrya* (Caesalpineae).

The ovules arise from the hypodermal layer at the margins of the folded legume, and are possibly morphologically abaxial. They are more or less anatropous, being intermediate between the Caesalpineae and the Mimoseae and Papilionaceae. They are naked at fertilization.

In the ovule there is one hypodermal archesporial cell as in the Mimoseae and Caesalpineae. The parietal tissue is early destroyed. The epidermis over the parietal tissue is multiple by the time of fertilization, and is persistent.

The megaspore is possibly smaller than the microspore.

In the anther, the single primary sporogenous cell from the division of each archesporial cell divides twice to form the four microspore mother cells, in each sporangium.

In the ovule, the primary sporogenous cell functions as the megaspore mother cell. Megaspore formation is succedaneous.

There is discussion of the significance of the small number of mother cells in connection with the theory that pairing of chromosomes at meiosis is due to delayed split in the pre-meiotic mitosis.

The approximately equal choice of the distal or proximal megaspore for functioning is unique among the Leguminosae.

The general and indefinite sterility is influenced by climate to different degrees in the stamens and legumes.

The symbols "ϕ" and "2ϕ" are adopted for the chromosome numbers of any species, "n" being reserved for the basic number of the genus. The number ϕ = 13 is confirmed, and counts are made of 2ϕ = 26. There are size differences in the chromosomes some of which may have trabants. The anaphase split in mitosis is clearly shown. The possibility that the species is a secondary polyploid is discussed.

The general conclusion will be reserved till the completion of the inquiry in a paper now almost ready for publication.

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Notes on the Illustrations.

All text-figures have been prepared from camea-lucida drawings. Of the figures on Plate vii, numbers 3 and 13-17*b'* are from camera-lucida drawings, the remainder being from photographs. All photography is the work of the writer except the enlarged print for figure 1 on the Plate (Mr. H. G. Gooch).

Annotations have been standardized throughout the Text-figures and the Plate-figures. A few special letterings are given in the legends and explanations. The following are the standard letterings:

a, archesporium; *ae*, appressed epidermes; *de*, multiple epidermis; *f*, filament (stamen), funiculus (ovule); *ii*, inner integument; *mb*, marginal bundles; *mc*, mother cell; *mr*, midrib; *oi*, outer integument; *p*, parietal tissue or layer(s); *pp*, primary parietal cell; *ps*, primary sporogenous cell; *s*, sporogenous cell(s); *SP*, functional spore; *sp*, non-functional spore; *t*, tapetum.

All magnifications were obtained by measurement.

EXPLANATION OF PLATE VII.

1.—Raceme at anthesis, showing proterogyny and exsertion of the styles. About natural size.

2.—Tip of a style with two pollinia projecting from the shallow stigmatic cup. × 380 (approx.).

3.—Tip of style with germinating pollinium and tubes from a second pollinium. Note shallow cup of stigma, and the thick cuticle (*ct.*) not in the cup. × 380 (approx.).

4.—Transverse section of a young pod between the seeds. The thickening on the collenchyma is not yet laid down. The midrib (lower end of figure) is beginning to be divided by parenchyma. *pce,* secondary parenchyma along epidermal line at the marginal side; *pcm,* secondary parenchyma dividing the midrib; *cc,* collenchyma cells; *l.* edge of loculus. × 410.

5 and 6.—Transverse sections of marginal and midrib edges of a full size pod. Collenchyma fully formed. Lettering as in Fig. 4. × 410.

7.—Longitudinal section of a young legume showing sterility in ovules about stage of formation of megaspores. × 158.

8.—Oblique transverse section of a young legume showing sterility in ovules that had attained nearly full size. × 150.

9.—Transverse section of a young legume before appearance of ovule primordia. × 220.

10.—Transverse section of a young legume showing the ovule primordia (*ov*) arising in the hypodermal layer. A and B, possible positions of the margins of the legume. × 220.

11 and 12.—Longitudinal sections of the marginal (adaxial) and midrib (abaxial) sides of a young legume showing the presence and absence respectively of appressed epidermes. About the age of the legume shown in Figure 9. × 220.

13.—Longitudinal section of a nucellus just before fertilization, showing starch (refringent) in the nucellar cells, and the multiple epidermis. × 540.

14.—Oblique longitudinal section of a young ovule with the nucleus of the functional spore (distal) in prophase. × 440.

15.—Anaphase of the homotypic division in the ovule. The distal spindle is horizontal, an exceptional case. In the distal cell only one group of chromosomes is shown in polar view with the anaphase split visible. × 2130.

16*a, b.*—Prophase nuclei of the two-nucleate stage of an embryo sac, showing 13 chromosomes each. The split and trabants are visible in some cases. Note the difference in the size of the chromosomes. Starch grains are in the cytoplasm. × 1060.

17*a, b, b'*.—Telophase nuclei of the third division in an embryo sac. Split and trabants visible in some chromosomes. 17*a* is the distal end in which the two darker nuclei are incomplete, three and one chromosomes belonging to the right and left nuclei being on the next section. Otherwise there are 13 chromosomes in each of the eight nuclei. Note the incipient wall formation between the left hand pair of nuclei in both groups. *b'* is the fourth nucleus from 17*b*. Starch grains present. × 2030.